ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 2008, p. 2270–2272 Vol. 52, No. 6
0066-4804/08/$08.00+0 doi:10.1128/AAC.00037-08

Genetic Environment of 16S rRNA Methylase Gene *rmtD*▽

Yohei Doi,[1]* Jennifer M. Adams-Haduch,[1] and David L. Paterson[1,2]

Division of Infectious Diseases, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania,[1] and University of Queensland, Royal Brisbane and Women's Hospital, Brisbane, Australia[2]

Received 9 January 2008/Returned for modification 24 February 2008/Accepted 24 March 2008

The genetic environment of the 16S rRNA methylase gene *rmtD* was investigated. *rmtD* was flanked by a novel IS*CR* motif located downstream of class I integron In*163* in the original *Pseudomonas aeruginosa* strain. *rmtD* found in *Klebsiella pneumoniae* appeared to have been mobilized from *P. aeruginosa* by an IS*26*-mediated event.

rmtD is one of the recently discovered 16S rRNA methylase genes that confer high-level aminoglycoside resistance (2). It was initially identified in *Pseudomonas aeruginosa* from Brazil (3). *rmtD* has since been found in *Klebsiella pneumoniae* and other species in the family *Enterobacteriaceae* as well (6, 14), raising questions as to how the gene was mobilized across the species. In the present study, we analyzed the nucleotide sequences surrounding *rmtD* in these strains in an attempt to answer this question.

P. aeruginosa PA0905 (3) and *K. pneumoniae* R2 (14), both known to carry *rmtD*, were used in the study. For *P. aeruginosa* PA0905, genomic DNA was digested with SpeI and ligated with pBC-SK(−) (Stratagene, La Jolla, CA). For *K. pneumoniae* R2, plasmid DNA was digested with EcoRI, PstI, or Sau3AI and ligated with the same cloning vector. *Escherichia coli* DH10B was then transformed with the ligated products by electroporation. Transformants that possessed recombinant plasmids encoding *rmtD* were selected on Luria-Bertani agar plates containing chloramphenicol (25 μg/ml) and amikacin (50 μg/ml). As a result, *rmtD*-containing sequences stretching a total of 17.9 kb and 9.1 kb were obtained from *P. aeruginosa* PA0905 and *K. pneumoniae* R2, respectively. Sequencing of the nucleotides was performed with an ABI 3100 instrument (Applied Biosystems, Foster City, CA).

A schematic presentation of the genetic environment of *rmtD* from the two strains is shown in Fig. 1. In *P. aeruginosa* PA0905, *rmtD* was located downstream of In*163*, a class I integron which has been reported in an amikacin-susceptible *P. aeruginosa* strain from Brazil (1). As in the original report, the integron contained three antimicrobial resistance gene cassettes, *aacA4*, bla_{OXA-56}, and *aadA7*, and was interrupted by *tnpA*, a putative transposase gene. The transposase shared 97% identity with that of IS*Pa21* reported in *P. aeruginosa* (10) and was accompanied by 13-bp perfect inverted repeats, IRL and IRR. The integrase gene *intI1* of In*163* was interrupted by *orf102*, which had no significant similarity with known sequences. However, *orf102* was accompanied by a putative 59-base element and thus may constitute a gene cassette. Since the initial report of In*163* did not contain the *intI1* sequence, it is not clear whether the defective gene is a common feature of this particular integron. *rmtD*, a putative tRNA ribosyltransferase gene, and truncated *groEL*, which likely originally encoded a heat shock protein, were located downstream of the 3′ conserved segment of In*163*. A putative tRNA ribosyltransferase gene has been reported to be located upstream of *rmtA*, the other 16S rRNA methylase gene found in *P. aeruginosa* (13). Though *rmtD* and *rmtA* share only 40% identity at the amino acid level, this similarity in alignment suggests that they may be derived from closely related ancestral species. This structure was bounded by two copies of *orf494* in the same direction. The deduced amino acid sequence of *orf494* was 96% identical to that of the putative transposase gene constituting IS*CR3* and found adjacent to the 16S rRNA methylase gene *rmtB* in *Serratia marcescens* and *E. coli* (5). IS*CR*s are a group of IS*91*-like elements that are implicated in the accumulation of various antimicrobial resistance genes (12; http://www.cardiff.ac.uk/medic/aboutus/departments/medicalmicrobiology/genetics/iscr/iscr_elements.html).

Orf494 possessed all the key amino acid motifs that are conserved among the transposases constituting IS*CR*s. In addition, both copies of *orf494* accompanied the consensus sequence for the 3′ ends of IS*CR*s (*ori*IS), 5′-GCGTTTGAACT TCCTATACXX-3′, 224 bp downstream of their 3′ ends. Thus, *orf494* and its flanking sequences likely represented a novel IS*CR* element, here designated IS*CR14*. In fact, *groEL* was truncated at the 5′ end by a short punctuated inverted repeat of 4 bp, which may represent the 5′ end of the IS*CR* (*ter*IS). *ter*IS for the other copy of *orf494*, which likely played a role in mobilizing In*163* adjacent to *rmtD*, could not be identified within the sequence studied. *sul1* was located downstream of the second copy of *orf494*, followed by part of Tn*5664* that likely originated from *Corynebacterium* species and contained *cmx*, a chloramphenicol export protein gene (11). The strain in which In*163* was originally described likely did not possess *rmtD* downstream of the integron, given its susceptibility to amikacin (1). Additional sequence information from this strain may further clarify the role of IS*CR14* in forming the resistance gene cluster downstream of In*163*. In any case, the results suggest that IS*CR3*/*14* elements played a vital role in mobilizing *rmtB* and *rmtD* to gram-negative pathogens from yet-unidentified sources.

In *K. pneumoniae* R2, the sequence adjacent to *rmtD* was identical with that observed in *P. aeruginosa* PA0905. However, both copies of *orf494* were interrupted by the insertion se-

* Corresponding author. Mailing address: Division of Infectious Diseases, University of Pittsburgh Medical Center, Falk Medical Building Suite 3A, 3601 Fifth Avenue, Pittsburgh, PA 15213. Phone: (412) 648-6401. Fax: (412) 648-6399. E-mail: doiy@dom.pitt.edu.
▽ Published ahead of print on 7 April 2008.

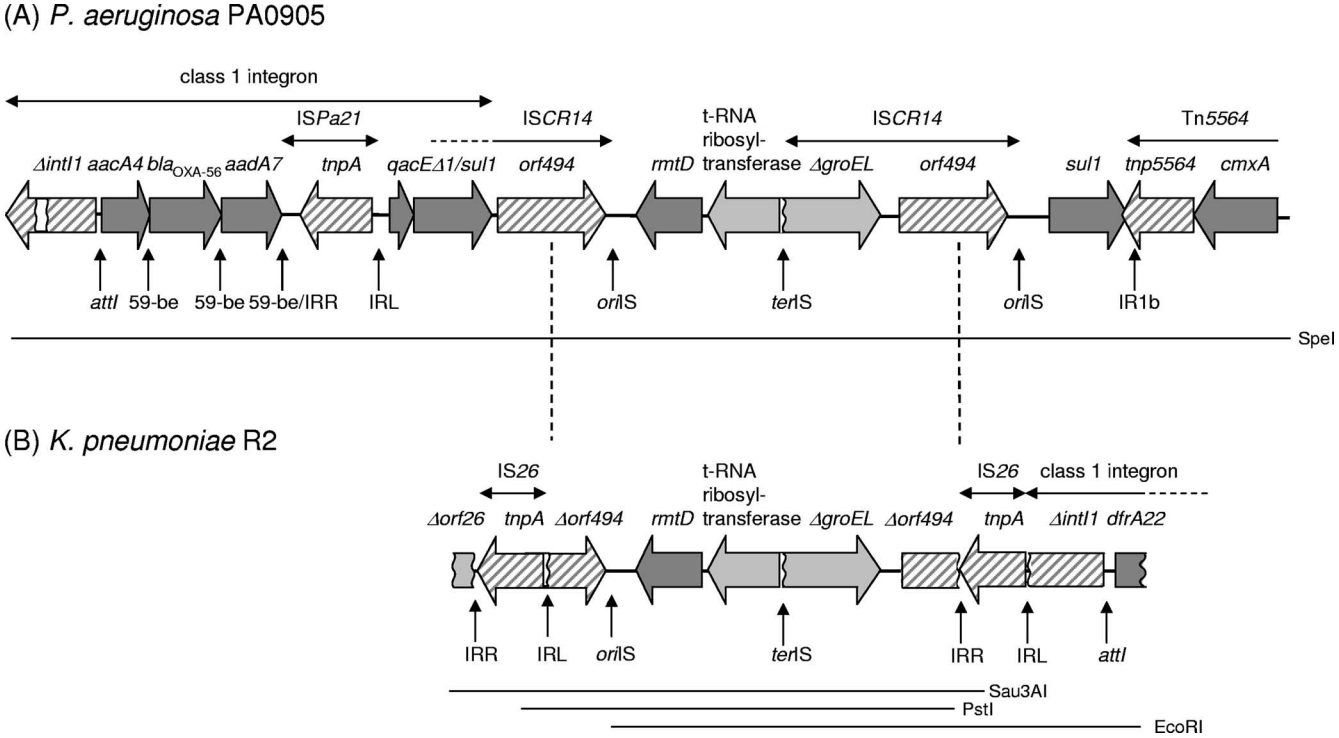

FIG. 1. Schematic presentation of the genetic environment of the *rmtD* gene. (A) The 17.9-kb section surrounding *rmtD* in *P. aeruginosa* PA0905. (B) The 9.1-kb section in *K. pneumoniae* R2. *attI*, attachment site of integrase; 59-be, 59-base element of gene cassette; IRR, right inverted repeat of IS*Pa21* or IS*26*; IRL, left inverted repeat of IS*Pa21* or IS*26*; *ori*IS, replication origin of IS*CR14*; *ter*IS, replication terminator of IS*CR14*; IR1b, 22-bp inverted repeat of Tn*5564*.

quence IS*26*. Interestingly, both copies of IS*26* were inserted at the same sequence, 5′-CGATCACC-3′, within each copy of *orf494*. Although IS*26* is not known to show marked target specificity (9), this particular sequence may have served as a recognition site for IS*26*. The 5′ end of the upstream copy of IS*26* was flanked by a class I integron. The 3′ end of *intI1* was truncated by the insertion of IS*26*. *dfrA22*, a trimethoprim resistance dihydrofolate reductase gene cassette, was located downstream of *intI1*. The 3′ end of the downstream copy of IS*26* was flanked by truncated *orf26*, which encoded an unknown protein and was reported in a conjugative plasmid encoding the 16S rRNA methylase gene *armA* (7). IS*26* is known to give rise to cointegrates in which the donor and target replicons are separated by two directly repeated IS copies (8, 9). It is unlikely that the two copies of IS*26* formed a composite transposon with each other, given the lack of direct repeat sequences and continuity of sequences at the outer ends. It may be possible, however, that additional IS copies are present further downstream of the integron which, along with the two copies described here, played a role in mobilization of *rmtD* from *P. aeruginosa* to *K. pneumoniae*.

Data in the literature to date suggest that *rmtD* is likely more prevalent in *P. aeruginosa* than *Enterobacteriaceae* in Brazil (4, 6). However, incorporation of *rmtD* by IS*26*-mediated recombinational events may facilitate future spread of the gene within *Enterobacteriaceae*. This has potential serious implications for the use of aminoglycosides in common infections caused by these species.

Nucleotide sequence accession numbers. The sequences reported in this study have been deposited with GenBank/EMBL/DDBJ under accession numbers DQ914960 and EU269034.

We thank Doroti de Oliveira Garcia and Flávia Rossi for provision of the strains.

Y.D. is supported by NIH training grant T32AI007333. D.L.P. is supported in part by NIH research grant R01AI070896.

REFERENCES

1. **Carvalho, A. P., R. M. Albano, D. N. de Oliveira, D. A. Cidade, L. M. Teixeira, and A. Marques Ede.** 2006. Characterization of an epidemic carbapenem-resistant *Pseudomonas aeruginosa* producing SPM-1 metallo-β-lactamase in a hospital located in Rio de Janeiro, Brazil. Microb. Drug Resist. **12:**103–108.
2. **Doi, Y., and Y. Arakawa.** 2007. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin. Infect. Dis. **45:**88–94.
3. **Doi, Y., D. de Oliveira Garcia, J. Adams, and D. L. Paterson.** 2007. Coproduction of novel 16S rRNA methylase RmtD and metallo-β-lactamase SPM-1 in a panresistant *Pseudomonas aeruginosa* isolate from Brazil. Antimicrob. Agents Chemother. **51:**852–856.
4. **Doi, Y., A. C. Ghilardi, J. Adams, D. de Oliveira Garcia, and D. L. Paterson.** 2007. High prevalence of metallo-β-lactamase and 16S rRNA methylase coproduction among imipenem-resistant *Pseudomonas aeruginosa* isolates in Brazil. Antimicrob. Agents Chemother. **51:**3388–3390.
5. **Doi, Y., K. Yokoyama, K. Yamane, J. Wachino, N. Shibata, T. Yagi, K. Shibayama, H. Kato, and Y. Arakawa.** 2004. Plasmid-mediated 16S rRNA methylase in *Serratia marcescens* conferring high-level resistance to aminoglycosides. Antimicrob. Agents Chemother. **48:**491–496.
6. **Fritsche, T. R., M. Castanheira, G. H. Miller, R. N. Jones, and E. S. Armstrong.** 2008. Detection of methyltransferases conferring high-level resistance to aminoglycosides in Enterobacteriaceae from Europe, North America, and Latin America. Antimicrob. Agents Chemother. **52:**1843–1845.
7. **Golebiewski, M., I. Kern-Zdanowicz, M. Zienkiewicz, M. Adamczyk, J.**

Zylinska, A. Baraniak, M. Gniadkowski, J. Bardowski, and P. Ceglowski. 2007. Complete nucleotide sequence of the pCTX-M3 plasmid and its involvement in spread of the extended-spectrum β-lactamase gene $bla_{CTX-M-3}$. Antimicrob. Agents Chemother. **51:**3789–3795.

8. **Iida, S., B. Mollet, J. Meyer, and W. Arber.** 1984. Functional characterization of the prokaryotic mobile genetic element IS*26*. Mol. Gen. Genet. **198:**84–89.
9. **Mahillon, J., and M. Chandler.** 1998. Insertion sequences. Microbiol. Mol. Biol. Rev. **62:**725–774.
10. **Poirel, L., L. Brinas, N. Fortineau, and P. Nordmann.** 2005. Integron-encoded GES-type extended-spectrum β-lactamase with increased activity toward aztreonam in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **49:**3593–3597.
11. **Tauch, A., Z. Zheng, A. Puhler, and J. Kalinowski.** 1998. *Corynebacterium striatum* chloramphenicol resistance transposon Tn*5564*: genetic organization and transposition in *Corynebacterium glutamicum*. Plasmid **40:**126–139.
12. **Toleman, M. A., P. M. Bennett, and T. R. Walsh.** 2006. IS*CR* elements: novel gene-capturing systems of the 21st century? Microbiol. Mol. Biol. Rev. **70:** 296–316.
13. **Yamane, K., Y. Doi, K. Yokoyama, T. Yagi, H. Kurokawa, N. Shibata, K. Shibayama, H. Kato, and Y. Arakawa.** 2004. Genetic environments of the *rmtA* gene in *Pseudomonas aeruginosa* clinical isolates. Antimicrob. Agents Chemother. **48:**2069–2074.
14. **Yamane, K., F. Rossi, M. G. Barberino, J. M. Adams-Haduch, Y. Doi, and D. L. Paterson.** 2008. 16S ribosomal RNA methylase RmtD produced by *Klebsiella pneumoniae* in Brazil. J. Antimicrob. Chemother. **61:**746–747.